

What is claimed is:

1. A fluorescent protein which is derived from Green Fluorescent Protein (GFP)
or any functional GFP analogue and has an amino acid sequence which is
5 modified by amino acid substitution compared with the amino acid sequence
of wild type Green Fluorescent Protein said modified fluorescent protein
comprising:
 - i) an amino acid substitution at position F64;
 - ii) a single amino acid substitution at a position selected from the group
10 consisting of positions S65 and E222; and
 - iii) an amino acid substitution at position S175;wherein said modified GFP has a different excitation spectrum and/or
emission spectrum compared with wild type GFP.
- 15 2. A fluorescent protein according to claim 1, wherein the amino acid F at
position 64 has been substituted by an amino acid selected from the group
consisting of L, I, V, A and G.
3. A fluorescent protein according to claim 1, wherein the amino acid S at
20 position 175 has been substituted by an amino acid selected from the group
consisting of G, A, L, I and T.

4. A fluorescent protein according to claim 1, wherein the amino acid S at position 65 has been substituted by an amino acid selected from the group consisting of G, A, L, C, V, I and T.
- 5 5. A fluorescent protein according to claim 1, wherein the amino acid E at position 222 has been substituted by an amino acid selected from the group consisting of G, A, V, L, I, F, S, T, N and Q.
- 10 6. A fluorescent protein according to claim 1 selected from F64L-S175G-E222G-GFP and F64L-S65T-S175G-GFP.
- 15 7. A fluorescent protein according to claim 1, further comprising an amino acid sequence which is modified by amino acid substitution compared with the amino acid sequence of wild type Green Fluorescent Protein having the sequence: SEQ ID No.2.
8. A fluorescent protein derived from Green Fluorescent Protein (GFP) and having the amino acid sequence as set forth in SEQ ID No.3.
- 20 9. A fluorescent protein derived from Green Fluorescent Protein (GFP) and having the amino acid sequence as set forth in SEQ ID No.4.
10. A fusion compound comprising a protein of interest fused to a fluorescent protein said fluorescent protein being a modified protein according to claim 1.

11. A nucleic acid molecule comprising a nucleotide sequence encoding a fluorescent protein which is derived from Green Fluorescent Protein (GFP) or any functional GFP analogue and has an amino acid sequence which is modified by amino acid substitution compared with the amino acid sequence of wild type Green Fluorescent Protein said modified fluorescent protein comprising:
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- i) an amino acid substitution at position F64;
 - ii) a single amino acid substitution at a position selected from the group consisting of positions S65 and E222; and
 - 10 iii) an amino acid substitution at position S175;
- wherein said modified GFP has a different excitation spectrum and/or emission spectrum compared with wild type GFP.
- 15 12. A nucleic acid molecule according to claim 11 encoding a fluorescent protein having an amino acid sequence which is modified by amino acid substitution compared with the amino acid sequence of wild type Green Fluorescent Protein having the sequence: SEQ ID No.2.
- 20 13. A nucleic acid molecule according to claim 11 encoding a fluorescent protein having an amino acid sequence selected from the group consisting of SEQ ID No.3 and SEQ ID No.4.

14. A nucleic acid molecule comprising a nucleotide sequence encoding a fusion protein, wherein said fusion protein further comprises a protein of interest fused to a fluorescent protein of claim 1.
- 5 15. An expression vector comprising suitable expression control sequences operably linked to a nucleic acid molecule according to claim 11.
16. A host cell transformed or transfected with a DNA construct comprising an expression vector according to claim 15.
- 10 17. The host cell according to claim 16 wherein said host cell is selected from the group consisting of a mammalian cell, a bacterial cell, a yeast cell and an insect cell.
- 15 18. A method for preparing a Green Fluorescent Protein (GFP) or a functional GFP analogue according to the present invention said method comprising cultivating a host according to claim 16 and obtaining therefrom the polypeptide expressed by said nucleotide sequence.
- 20 19. A method of measuring the expression of a protein of interest in a cell which method comprises:
- i) introducing into a cell a nucleic acid molecule comprising a nucleotide sequence encoding a fluorescent protein which is derived from the Green Fluorescent Protein (GFP) or any functional GFP analogue

according to claim 1, said nucleic acid molecule being operably linked to and under the control of an expression control sequence which moderates expression of said protein of interest;

- ii) culturing said cell under conditions suitable for the expression of said protein of interest; and
- iii) detecting the fluorescence emission of said Green Fluorescent Protein (GFP) or a functional GFP analogue as a means of measuring the expression of said protein of interest.

20. A method of determining the cellular and/or extracellular localisation of a protein of interest which method comprises:

- i) introducing into a cell a nucleic acid molecule comprising a nucleotide sequence encoding a fluorescent protein which is derived from the Green Fluorescent Protein (GFP) or any functional GFP analogue of claim 1 fused to a nucleotide sequence encoding a protein of interest, said nucleic acid molecule being operably linked to and under the control of a suitable expression control sequence;
- ii) culturing said cell under conditions suitable for the expression of said protein of interest; and
- iii) determining the cellular and/or extracellular localisation of said protein of interest by detecting the fluorescence emission by optical means.

21. A method of comparing the effect of one or more test substance(s) on the expression and/or localisation of one or more different protein(s) of interest in a cell which method comprises:
- 5 i) introducing into a cell a nucleic acid molecule comprising a nucleotide sequence encoding a Green Fluorescent Protein (GFP) or a functional GFP analogue of claim 1, said nucleic acid molecule being operably linked to and under the control of a first expression control sequence;
 - 10 ii) culturing said cells under conditions suitable for the expression of said protein(s) of interest in the presence and absence of said test substance(s);
 - iii) determining the expression and/or localisation of said protein(s) of interest in said cells by detecting the fluorescence emission by optical means; and
 - 15 iv) comparing the fluorescence emission obtained in the presence and absence of said test substance(s) to determine the effect of said test substance(s) on the expression and/or localisation of said protein(s) of interest.
22. The method of claim 21 wherein said nucleic acid molecule of said introducing step is fused to a nucleotide sequence encoding a first protein of interest.
23. The method of claim 21, wherein said introducing step further includes at least one different nucleic acid molecule encoding a protein reporter molecule

optionally fused to a different protein of interest, each said nucleic acid molecule being operably linked to and under the control of a second expression control sequence wherein said protein reporter molecule has or is capable of generating an emission signal which is spectrally distinct from that of said Green Fluorescent Protein (GFP) or functional GFP analogue;

24. The method according to claim 21 wherein samples of said cells in a fluid medium are introduced into separate vessels for each of said test substances to be studied.

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